the C-terminus of VDAC1 is exposed to the cytosolic side, we expect to lose the His tag upon DEVD cleavage. As result only the HA can be detected at mitochondrial level. Conversely if the C-terminus is oriented towards the IMS, the DEVD is not cleavable and both tags will be detected in mitochondria. Our results by confocal microscopy and appropriate controls with other membrane-oriented constructs showed that this strategy is able to define the sidedness of the VDAC pore.

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2772-Plat

Deciphering the Interaction of FLAP and 5LO

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Leukotrienes are pro-inflammatory lipid mediators involved in chronic inflammatory diseases like asthma and atherosclerosis. Different enzymes and proteins are involved in the leukotriene biosynthesis pathway that stems from oxygenation of arachidonic acid (AA) by 5LO (5-lipoxygenase). FLAP (5-lipoxygenase activating protein) is an integral membrane protein, belonging to the MAPEG (Membrane Associated Proteins in Eicosanoid and Glutathione Metabolism) family. Till now, there is no prominent evidence for any biochemical or enzymatic activity for FLAP, except involvement as an ''activator'' in leukotriene biosynthesis. The hypothesis is that, on increase in intracellular calcium concentration, 5LO moves from the cytosol to the nuclear membrane and localizes near FLAP. The AA is then transferred from the nuclear membrane to 5LO by homo-trimeric FLAP. Then 5LO converts the AA to leukotriene A4. This interaction between FLAP and 5LO is ambiguous due to intricate mechanisms which occur at the interface of the nuclear membrane and also between an enzyme and an integral membrane protein with no documented function. To understand the function and involvement of FLAP in leukotriene biosynthesis in vitro, we employ soluble phospholipid bilayers called ''Nanodiscs'' which mimic a membrane environment. The nanodisc will act as a stable platform for structural and functional characterization of the interaction between 5LO and FLAP. After initial studies of the Ca^{2+}dependent recruitment of 5LO to empty nanodiscs as well as the reconstitution of FLAP into nanodiscs, the entire complex 5LO-FLAP-nanodisc can be targeted. We employ biochemical assays to characterize the interactions and transmission electron microscopy (single particle analysis) to create a 3D model of the functional complex of 5LO and FLAP. Here, we portray our outcomes from the above mentioned subprojects encompassed in understanding the interaction of 5LO and FLAP.

2773-Plat

Membrane Anchoring Amplifies Osmosensing by the Inner Membrane Receptor EnvZ

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A fundamental question in signalling biology is how do receptors transmit the effects of extracellular stimuli across membranes? Transmembrane segments that connect a receptor extracellular domain to its intracellular domain are often considered to be critical elements for transmitting these signals. The dynamics of receptor signalling is not clearly understood, as there are few biophysical probes to directly monitor transmembrane regions. Amide Deuterium Exchange Mass Spectrometry (HDXMS) provides an excellent method to describe the conformational dynamics of membrane bound proteins. Our recently published studies with the cytoplasmic domain of the bacterial inner membrane osmosensor kinase EnvZ (EnvZc) have revealed the molecular basis for osmosensing through modulation of helix-coil transitions in a critical four helix bundle subdomain (Wang, LC, Morgan, L, Godakumbura, P, Kenney, LJ and Anand, GS EMBO J. 31(11):2648-59, 2012). This study highlighted the importance of local folding-unfolding equilibria in the functioning of EnvZ signalling. Most surprising was that a cytoplasmic deletion fragment of EnvZ lacking the transmembrane helices was able to fully rescue the osmosensing function in a strain lacking the envZ gene. This immediately raised the question as to what is the function of membrane anchoring? Our preliminary results indicate that the helix-coil transitions and associated local unfolding we observed in EnvZc also occur in transmembrane helix regions in EnvZ. Conformational dynamics of full-length EnvZ embedded within nanodiscs indicate that the osmosensing core of EnvZ shows greater deuterium exchange compared to EnvZc, suggesting a regulatory role of the membrane in modulating the autokinase activity of EnvZ. Our results reveal that membrane anchoring is responsible for 'damping down' the helix-coil transitions and likely increases the sensitivity range for the receptor. These results are broadly relevant to all receptors. Supported by Mechanobiology RCE and VA-1IOBX000372 to LJK.

Platform: Protein-Nucleic Acid Interactions II

2774-Plat

PICH: A DNA Translocase Essential for Resolving Anaphase Bridged DNA

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PICH (PLK1-Interacting Checkpoint Helicase) is a recently identified member of the RAD54 subgroup of SNF2 family proteins. PICH localizes to so-called ultra-fine anaphase bridges (UFBs) in mitosis alongside a multi-protein complex of DNA repair proteins including BLM, the Bloom's syndrome gene product. Very little is known about the function of PICH or how it is recruited selectively to UFBs. Nevertheless, depletion of PICH results in genomic instability, including an elevated frequency of sister chromatid exchanges, micronuclei and loss of heterozygosity. using a combination of microfluidics, single-molecule fluorescence microscopy and optical tweezers, we have defined the properties of PICH in an in vitro model of an anaphase bridge. We show that PICH binds with a remarkably high affinity to dsDNA and displays ATP-dependent dsDNA translocase activity. The application of stretching forces to the DNA, which mimics the effects of the mitotic spindle on a UFB, enhances the binding of PICH to dsDNA, but also serves to diminish stretching-induced DNA melting. Based on our findings, we suggest that PICH plays several roles in the development and processing of UFBs: (i) to recognize and bind to dsDNA exposed by the mitotic spindle force-induced unwrapping of nucleosomes, (ii) to help expel exclusively these unwrapped nucleosomes, (iii) to stabilize stretched dsDNA, and (iv) to recruit the DNA repair machinery required for UFB resolution in anaphase.

2775-Plat

Computational Study of RNA Translocation in a Hexameric Helicase

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Hexameric helicases are ATP-driven molecular motors that participate in important genetic processes. A particularly interesting helicase is E. coli transcription termination factor Rho, which translocates towards the 3'-end of nascent transcript. It is still an open question of how the ATP hydrolysis cycle is coupled to RNA translocation in Rho. We present results from all-atom molecular dynamics simulations that studied the conformation transitions and the corresponding energy landscape in the hydrolysis cycle based on the available crystal structure of Rho (Nathan D. Thomsen and James M. Berger. 2009, Cell, 139:523-534). We define collective variables involving the conformations of key residues at the monomer-monomer interface in different ATP binding states. The simulations reveal how interface conformational changes propagate around the circular helicase and regulate RNA translocation along the central channel in a collaborative manner. Monomers change their relative positions along the translocation direction based on the ATP binding states. The suggested allosteric inter-monomer communication in Rho is also revealed by network analysis based on cross-correlation of protein motion. Lys326 in each monomer is crucial in ratcheting the RNA and its movement is coupled to the ATP binding state. Arg269 participates directly in linking monomer-monomer communication with the ATP binding state. The simulations further demonstrate the influence of different RNA sequences (poly(U) and poly(C)). Our study, which elucidates the structure-function relationship in Rho, can be extended to other hexameric helicase systems, such as E1 and DnaB, whose crystal structures in complex with substrates are available.

2776-Plat

DNA Scanning Mechanism of a Translocating Motor Protein

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Unrepaired DNA breaks can lead to genomic instability or cell death. For repair by the ubiquitous homologous recombination pathway, broken ends are first processed to produce a 3'-ssDNA overhang. In Bacillus subtilis, this reaction

is catalysed by AddAB helicase-nuclease complexes; motor proteins that unwind the DNA duplex and degrade the nascent single-strands in a manner regulated by specific single-stranded DNA sequences called Chi recombination hotspots (Yeeles and Dillingham, 2007; Yeeles et al., 2011). We have used Magnetic Tweezers to investigate the real-time dynamics of AddAB translocation on dsDNA and the effect of recombination hotspot recognition on this process. AddAB translocation traces showed a complex appearance with variable velocities between 200-400 bp/s at room temperature. We found that AddAB was prone to stochastic pausing in areas which contained many Chi-like sequences. Experiments using an AddAB mutant that is unable to recognize Chi strongly suggest that this pausing is due to transient recognition of Chi-like sequences and highlight the antagonistic nature of DNA translocation and sequence specific DNA recognition activities. Experiments using substrates containing bona fide Chi sequences showed that AddAB also pauses at Chi, but these events are longer and not exponentially distributed, suggesting a multistep process. We propose a model for the recognition of Chi and Chi-like sequences to explain the origins of this pausing behavior during failed or successful hotspot recognition.

Yeeles, J. T., and Dillingham, M. S. (2007). A dual-nuclease mechanism for DNA break processing by AddAB-type helicase-nucleases. J Mol Biol 371, 66-78.

Yeeles, J. T., van Aelst, K., Dillingham, M. S., and Moreno-Herrero, F. (2011). Recombination hotspots and single-stranded DNA binding proteins couple DNA translocation to DNA unwinding by the AddAB helicase-nuclease. Molecular Cell 42, 806-816.

2777-Plat

Single-Molecule Studies of Nucleosome Translocation by the ACF Chromatin Remodeling Complex

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The packaging of eukaryotic genomes as chromatin restricts access to the DNA for critical processes such as replication, transcription, recombination, and repair. Thus, eukaryotic cells depend on a dynamic balance between genome compaction and access facilitated in part by chromatin remodeling enzymes (remodelers). Remodelers read epigenetic marks such as histone modifications and use the energy of ATP hydrolysis to assemble, disassemble, reposition, and modify the composition of nucleosomes. Single-molecule techniques enable characterization of transient intermediates formed during the remodeling reaction and can therefore uncover previously unobtainable insights into the mechanisms of chromatin remodeling.

In this study, we developed a single-molecule fluorescence resonance energy transfer assay to study the dynamics of chromatin remodeling by human ACF, a prototypical member of the ISWI family of remodelers. ISWI remodelers are endowed with the ability to create regularly-spaced nucleosome arrays characteristic of transcriptionally silent heterochromatin.

With the addition of ACF and ATP, nucleosomes exhibit gradual translocation along the DNA interrupted by kinetic pauses after approximately seven or three base pairs (bp) of translocation, thereby dividing the remodeling process into alternating translocation phases and pause phases. Moreover, we found that ACF is a highly processive and bidirectional nucleosome translocase capable of sliding a nucleosome back-and-forth for an average of 200 bp before dissociating.

The nucleosome spacing activity of ISWI remodelers is regulated by two substrate features: (1) length of linker DNA, and (2) histone H4 N-terminal tail. Shortening the linker DNA or altering the H4 tail decrease the catalytic activity of ISWI remodelers with little effect on binding affinity. We discovered that the catalytic defects associated with these substrate modifications are attributed solely to changes in the pause phases and not the translocation phases, providing new insights into how nucleosomal features regulate chromatin remodelers.

2778-Plat

Spoiiie Mechanism of Directional Translocation Involves Target Search Coupled to Allosteric Motor Activation

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SpoIIIE/FtsK are membrane-anchored, ATP-fueled, directional motors responsible for chromosomal segregation in bacteria. Directionality in these motors is governed by interactions between specialized sequence-recognition modules (SpoIIIE-γ/FtsK-γ) and highly-skewed chromosomal sequences (SRS/KOPS). using a novel combination of bulk and single-molecule methods we dissect the series of steps required for SRS localization and motor activation. First, we demonstrate that SpoIIIE/DNA association kinetics are sequence-independent with binding specificity being uniquely determined by dissociation. Next, we show by single-molecule and modeling methods that hexameric SpoIIIE binds DNA non-specifically and finds SRS by an ATP-independent target search mechanism, with ensuing oligomerization and binding of SpoIIIE-γ to SRS triggering allosteric motor activation. We propose a new model that provides an entirely novel interpretation of previous observations for the origin of SRS/KOPS-directed translocation by SpoIIIE/FtsK.

2779-Plat

E. Coli RNA Polymerase Searches for Promoters through 3D Diffusion

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Gene expression, DNA replication, and genome maintenance all start with site-specific DNA binding proteins, which must recognize specific targets from among a vast excess of nonspecific DNA. For example, to initiate transcription, E. coli RNA polymerase (RNAP) must locate promoter sequences, which comprise <2% of the bacterial genome. This promoter search problem remains one of the least understood aspects of gene expression, largely due to the transient nature of intermediates involved in the search process. Here we use single-molecule microscopy to visualize RNAP in real time as it searches for promoters, and we develop a theoretical framework that allows us to analyze target searches at the submicroscopic scale based on single-molecule promoter association rates. Contrary to long-held assumptions, we demonstrate that the promoter search by E. coli RNAP is dominated entirely by 3D diffusion, which has direct implications for understanding how E. coli RNAP and other proteins locate their targets within physiological settings.

2780-Plat

The ATP Hydrolysis Cycle and the Corresponding Motion of RecA Filament on Single-Stranded DNA

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RecA is involved in DNA repair mechanism in bacteria by catalyzing homologous strand exchange reaction. RecA forms a filament on a single-stranded DNA made at the double-strand breaks. RecA adopts either a stretched (active) structure with ATP as a cofactor, or a compressed (inactive) structure with ADP. At the filament end, a monomer is allowed to dissociate after ATP hydrolysis and to rebind. We developed a single-molecule fluorescence assay to investigate how the ATP hydrolysis is coupled with the dynamics of the RecA filament. We dissected the intermediate steps of ATP hydrolysis and discovered that a monomer at the filament end dissociated upon the hydrolysis of ATP and not after the release of Pi. Interconversion of the structure between stretched- and compressed forms was achieved via cooperative structural change of a group of neighboring monomers. Owing to this cooperativity, an internal RecA monomer continuously consumes and rapidly refreshes ATP molecule without changing its stretched conformation. Based on our observation, we suggest a model of ATP hydrolysis cycle of the RecA in the filament.

2781-Plat

How DNA-Binding Proteins find their Target Sites in Human Cells

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Precise and timely expression of specific genes is fundamental for the viability of any organism. Specific DNA-binding proteins (transcription factors, TFs) transiently associate to promoter sequences on the genome to activate or repress transcription of specific genes. The way TFs explore the nuclear space, find, and bind to their target sequences is the key to decipher transcriptional control but is still subject of debate.

We exploit a live-cell model system to identify what factors primarily orchestrate TFs target search. Our assay is based on human cells containing, at a single locus, specific DNA binding sites ('target') for an exogenous DNA-binding protein (TetR, 'searcher'). First, single-particle-tracking experiments revealed that individual TetR proteins move in a very composite way. They transiently interact with nonspecific DNA sequences, via their DNA-binding-domain, with residence times ranging from hundreds of millisecond up to few seconds. Furthermore, a subset of proteins shows confinement within micron-sized regions